View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Digital.CSIC

Constitutive defenses and damage in Sitka spruce progeny obtained from crosses between white pine weevil resistant and susceptible parents

Xoaquín Moreira [1,4], René I. Alfaro[2], John N. King[3]

[1]Centro de Investigación Forestal de Lourizán – Unidad Asociada MBG-CSIC, Apdo. 127, 36080 Pontevedra, Galicia, Spain. Email: xmoreira@mbg.cesga.es

[2]Pacific Forestry Centre, Canadian Forest Service, 506 West Burnside Road, Victoria, BC,V8Z 1M5, Canada. Email: ralfaro@pfc.forestry.ca

[3]British Columbia Ministry of Forests and Range, P.O. Box 9519 Stn. Prov. Govt., Victoria, BC, V8W 9C2, Canada. Email: John.King@gov.bc.ca

[4]Corresponding author:

Email: xmoreira@mbg.cesga.es

Phone Number: +34986805078

Fax Number: +34986856420

Number of total pages (including references, figures and tables): 29

Number of Tables: 4

Number of Figures: 2

Abstract

Attack by the white pine weevil has notably reduced Sitka spruce productivity in British Columbia (Canada) and western USA. By the 1970s, the British Columbia Ministry of Forests established provenance trials of Sitka spruce with the objective of detecting usable genetic resistance to weevil. These early trials reported significant weevil resistance and allowed the production of the first (F1) controlled-cross progeny generation with demonstrable weevil resistance (R) or susceptibility (S). This study reports results of the screening for weevil resistance and the levels of constitutive defenses of this F1 Sitka spruce progeny. Progeny from resistant parents (R × R progeny) sustained significantly fewer weevil attacks than progeny from susceptible parents (S × S progeny), or progeny with one resistant and one susceptible parent (R × S progeny). Individual and family heritability estimates of the weevil resistance were 0.5 and 0.9, respectively. Constitutive defenses, measured by resin canal and sclereid cell density in the cortex, were significantly higher in R × R progeny than in R × S or S × S progeny. We observed a negative correlation between the percentage of trees attacked in each cross and the average density of the resin canals or sclereid cells for each cross.

Keywords: *Controlled-cross progeny; Picea sitchensis; Pissodes strobi; resin canals; sclereid cells*; tree resistance

Introduction

The white pine weevil, *Pissodes strobi* Peck (Coleoptera: *Curculionidae*), is a serious forest pest in young pine and spruce plantations in much of Canada and the northern USA (Mitchell et al. 1990; Alfaro et al. 1995). Adults emerge in early spring to begin feeding and egg-laying in the secondary phloem of the one-year-old terminal leader of young trees (Silver 1968). Upon hatching, larvae mine downward consuming the secondary phloem, girdling and killing the leader (Silver 1968). This pest causes serious economic impact since leader damage cause large reductions in height growth and severe stem deformations, such as crooks and forks (Alfaro 1989).

In Western North America, this insect causes damage to Sitka spruce (*Picea sitchensis* (Bong.) Carr) (Alfaro et al. 2000), white spruce (*Picea glauca* (Moench) Voss) (He and Alfaro 2000), Engelmann spruce (*Picea engelmannii* Parry) (Mitchell et al. 1990) and interior spruce (*P. glauca* × *P. engelmanni*) (Taylor et al. 1994). Among these species, Sitka spruce is particularly vulnerable to weevil damage. During the past decades, thousands of hectares of young Sitka spruce were planted annually in Canada and the United States, but damage by the white pine weevil has forced managers to severely reduce the planting of this species. For example, in British Columbia (BC), Sitka spruce plantings have been reduced from a historic level of 10 million to fewer than one million trees annually (King and Alfaro 2009).

Several chemical, silvicultural and biological control measures have been tested against *P. strobi*, but none of them has been entirely effective (Alfaro et al. 1995). Genetic resistance offers a promising alternative for weevil management and successful spruce reforestation (Alfaro et al. 1996; King et al. 1997; Alfaro et al. 2004). Strong genetic variation in resistance, both among and within provenances, has been reported for Sitka spruce (Alfaro et al. 2008) and interior spruce (King et al. 1997). In the case of Sitka spruce, there has been already a substantial investment in genetic improvement in British Columbia, with the first provenance trials of Sitka spruce established at the beginning of the 1970s by the British Columbia

Ministry of Forests. Results of these early trials showed a notable variation in resistance to this weevil among spruce provenances (Alfaro and Ying 1990). Resistant provenances that were identified in these studies include the Haney provenance (Lower Fraser Valley), the Big Qualicum provenance (East Vancouver Island), and the Sitka-white spruce hybridization zone in the Skeena River area (Ying 1991; King et al. 2004; King and Alfaro 2009), all of them in British Columbia. This early work allowed the selection of a number of individual parent trees with high resistance (King and Alfaro 2009) and the production of a first (F1) progeny generation resulting from controlled-crosses using identified resistant (R) and susceptible (S) parents. This F1 progeny is the subject of this study. The establishment of this F1 progeny trial provided the opportunity to investigate the inheritance of resistance in Sitka spruce, the resistance rankings of the controlled-cross F1 progeny generation, and the anatomical traits that may act as defensive mechanisms against the weevil.

Resistance to insect herbivory is closely linked to the presence of evolved defensive mechanisms which are commonly subjected to strong genetic control (Núñez-Farfán et al. 2007). Defensive traits associated with phloem feeding insects, such us chemical composition of tree resin, the amount of resin flow, the number and density of resin canals and sclereid cell density have been shown to be under genetic control in several conifer species (Wainhouse and Ashburner 1996; Roberds et al. 2003; Rosner and Hannrup 2004; Gerson et al. 2009). The constitutive resin canal system present in the bark of many conifer species is regarded as the first stage and one of the most important defense mechanisms against stem-invading insects (Tomlin and Borden 1994; Alfaro 1995; Alfaro et al. 2004; Franceschi et al. 2005) and it has been reported to be a potential trait for selection and breeding (Alfaro et al. 1997; 2004; King and Alfaro 2009; King et al. 2011). The resin canal system fundamentally acts as physical barrier against herbivore insects, with the resin contained in the canals acting as a chemical defense, causing repellency and toxicity, which alters the feeding behavior of herbivores and increases larval mortality (Phillips and Croteau 1999; Trapp and Croteau 2001). Sclereid cells

can be found in the secondary phloem, forming small and hard bundles that act as a physical barrier to penetration by phloem invading organisms (Cassab 1998). Alfaro et al. (2002) and King et al. (2011) presented evidence of higher number of sclereid cells in trees from open-pollinated spruce provenances with resistance to weevil than in susceptible provenances.

In this study we present results of the screening for weevil resistance of a F1 Sitka spruce controlled-cross progeny, aiming at determining the inheritance of resistance in this important forest species. We also studied the role of the constitutive defenses, measured by resin canal and sclereid cell density, in weevil resistance, in order to investigate the possibility of using these defensive traits in further screening for weevil resistance in Sitka spruce.

Materials and methods

Study area

The study area was located in southwestern Vancouver Island (British Columbia, Canada). The trial consisted of a controlled-cross F1 progeny trial of Sitka spruce near Jordan River (48˚25'N, 124˚01'W; 31 m a.s.l.; 3 km from sea). The climate in this area is Maritime variant 2 (biogeoclimatic zone CWHxm2) (Klinka et al. 1991), with annual precipitation of approximately 2,800 mm and mean annual temperature of 12.0ºC. Soil in this area is Humo-Ferric Podzol with brownish coloured B horizons at least 10 cm thick that are enriched with amorphous material composed of humified organic matter and iron (Soil Classification Working Group 1998).

Plant material and experimental design

The control-pollinated progeny trial was established using one-year-old container seedlings of Sitka spruce in the winter of 2004 planted at 2.5 m spacing and was located in a moderate to high weevil hazard area, in order to secure a favorable environment for weevil population development, which would result in effective resistance screening. Forty-two female and

twenty-eight male parents of three different provenances were crossed to generate 110 full-sib controlled-crossed, F1 progenies (not every male parent was crossed with every female parent) (Table 1). Individual parents for the F1 progenies were selected based on their weevil resistance rankings as determined in earlier screening studies of open-pollinated and clonal trials (Alfaro et al. 2008; King and Alfaro 2009). The selected parents belong to three provenances that differed in their resistance to weevil: parents from Big Qualicum River (BQ) and Haney (H) provenances, which are classified as resistant to weevil (R) based on earlier, well-replicated experiments (King and Alfaro 2009), and parents from Queen Charlotte Islands (QCI, these islands are now named Haida Gwaii), which are classified as weevil susceptible (S) based on known susceptibility and thus are used as "susceptible" controls (King and Alfaro 2009). Thus, in the F1 progeny trial we had six types of controlled-cross progenies based on parent provenance (H × H, H × BQ, BQ × BQ, as R × R crosses – the major part of the trial, H × QCI, BQ × QCI, as R × S crosses – to create segregating crosses, and QCI × QCI, as S × S crosses – used as susceptible controls) (Table 1). The trial was established in a randomized complete-block design with 30 blocks, each containing one tree from each controlled-cross. The total number of trees in the trial was 3300, corresponding to 110 controlled-cross progenies × 30 blocks.

Weevil resistance

A natural population of *P. strobi* caused sporadic low-level attacks in the controlled-cross progeny trial during the first three years after plantation (from 2004 to 2007). Each tree was scored as attacked (value = 1) or not attacked (value = 0) in September 2007. We considered an attack as successful when weevil oviposition and larval feeding resulted in the death of the leader. Dead leaders due to weevil were easily identified since they contained oviposition punctures and emergence holes of the new adults and the phloem was consumed. This 2004-

158 2007 weevil damage was identified as “Old Attack”. The 2007 survey indicated that only 0.5%
159 of the trees had sustained Old Attack. For this reason, in order to ensure sufficient population
160 pressure for effective screening, the weevil population in the trial was augmented using the
161 techniques described by Alfaro et al. (2008). Briefly, for weevil augmentation, weevils were
162 reared from infested leaders collected in late summer 2006 from infested regeneration near trial
163 site. Adult weevils were maintained in 5 gallon bucket in wire mesh cages with spruce
164 branches for food until the next spring. Water was provided by misting. In September 2007, 3-
165 4 weevils were released onto each tree in the trial.
166 Total height of each tree was assessed in September 2008, one year after weevil release.
167 For height measurement we excluded trees with dead or damaged leaders in any previous year.
168 Weevil damage of each tree was again assessed in September 2008 and 2009, one and two
169 years after the release of weevils into the plantation. Weevil damage in these years was
170 evaluated following the same procedure as for the measurement of the Old Attack. To calculate
171 a final measure of resistance level we noted the presence or absence of attack from 2004 to
172 2009, scoring each tree as attacked in any year (value = 1) or never attacked (value = 0).
173
174 **Constitutive defenses**
175 In order to explore the relevance of constitutive defenses in determining Sitka spruce resistance
176 to pine weevil, we studied a subsample of spruce seedlings within the trial. To avoid possible
177 modification of the shoot anatomy or induction of defenses by weevil attack, all leader samples
178 within a cross were chosen from trees that had escaped weevil attack (i.e., never attacked from
179 2004 to 2009). We sampled apical leader tissue in five replicates of eight controlled-cross
180 progenies from resistant (R × R) parents (four from H × H parent provenance and four from
181 BQ × BQ parent provenance), eight controlled-cross progenies derived from resistant and
182 susceptible parents (R × S) (four from H × QCI parent provenance and four from BQ × QCI

183 parent provenance), and four controlled-cross progenies from susceptible parents (QCI × QCI).
184 Total sample size was 100, corresponding to 20 controlled-cross progenies x 5 replicates.
185 In May 2010, terminal leaders (2009 growth) from these 100 trees were destructively
186 sampled. Total length and diameter of each terminal leader was measured in the lab. Samples
187 were immediately placed in glass vials and fixed in formalin acetic acid for 48 h, and then
188 transferred to 70 % EtOH for storage until sectioning and staining. Cross-sections, 90 µm
189 thick, were made using a sliding microtome. Sections were stained with 0.1% aqueous Safranin
190 according standard procedures (Ruzin 1999; Moreira et al. 2008). Photographs were taken with
191 a Leica Digital Sight DFC320, mounted on a Leica MS5 light binocular microscope at x10
192 magnification. Digital image analysis was used to measure constitutive defenses on a randomly
193 selected quarter of cross section of the leader shoot, with the Phloemalizer v.2.12 image
194 analysis software developed at the Pacific Forestry Centre (Victoria, BC, Canada).
195 The following variables were calculated for each cross section sample: (i) inner, outer
196 and total resin canal density, measured by the number of resin canals per square millimeter of
197 bark, and by the percentage of bark area occupied by them, and (ii) sclereid cell density,
198 determined by the number of groups of sclereid cells per square millimeter of bark. Inner resin
199 canals are the ring of large, uniformly distributed ducts closest to the cambium, and outer ducts
200 refer to all other ducts, which are generally smaller and located close to the edge of the cross
201 section (Figure 1). Additional descriptions of inner and outer resin canals can be found in
202 Tomlin and Borden (1994). Total resin canal density was the sum of inner and outer resin
203 canals. Sclereids occur throughout the cortex but are often associated with vascular bundles.
204 Cortical vascular bundles occur in the cortex of primary shoots of conifers and may or may not
205 be associated with lateral buds or needles. Inner and outer resin canals and groups of sclereid
206 cells are shown in the Figure 1.
207

Statistical analyses

Total tree height was analyzed for statistical differences by Block, Resistance class, Provenance and Cross using the PROC-MIXED procedure of the SAS System (Littell et al. 2006) using the following mixed model:

$$[1] \qquad Y_{ijkl} = \mu + B_i + R_j + P\,(R)_{kj} + C\,(P*R)_{lkj} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the continuous response variable tree height; μ is the general mean, B_i, R_j, P_k and C_l are the main effects of block i (i = 1 to 30), resistance class j (j = 1 to 3, corresponding to R × R, R × S and S × S crosses), provenance k (k = 1 to 3) and controlled-cross progeny l (l = 1 to 88), and ε_{ijkl} is the residual error. Provenance was nested within resistance, $P\,(R)_{kj}$, and controlled-cross was nested within provenance and resistance, $C\,(P*R)_{lkj}$. All these factors were considered fixed effects.

Weevil damage was modeled with PROC-GLIMMIX (binary distribution) according to:

$$[2] \qquad Y_{ijkl} = 1/(1+\exp(\mu + B_i + R_j + P\,(R)_{kj} + C\,(P*R)_{lkj})$$

where Y_{ijkl} is a binary response (attacked or not) and all other model terms are the same as those used for total tree height above.

Length and diameter of the terminal leaders and density of resin canals and sclereid cells were analyzed using the PROC-MIXED procedure of the SAS System (Littell et al. 2006) and the following mixed model:

$$[3] \qquad Y_{ijk} = \mu + R_i + P\,(R)_{ji} + C\,(P*R)_{kji} + \varepsilon_{ijk}$$

where Y_{ijk} is a continuous response variable for resin canal and sclereid density or for the percentage of resin canal area relative to bark area; μ is the general mean, R_i, P_j and C_k are the main effects of resistance level i (i = 1 to 3), parent provenance j (j = 1 to 3) and controlled-cross progeny k (k = 1 to 8), and ε_{ijk} is the residual error. Parent provenance was nested within resistance, and controlled-cross progeny was nested within provenance and resistance. All

these factors were considered fixed effects. Diameter of terminal leader was used as covariate in the analysis of resin canal and sclereid cell density.

When needed, normality was achieved by log-transformation (sclereid cell density) and by arcsin-transformation (resin canal density measured by the percentage of bark area occupied by them) of the original variables. When main effects were significant, differences among means were tested for significance using the LSMEAN statement of the SAS System (Littell et al. 2006).

Individual (h^2_i) and family (h^2_f) heritabilities for weevil resistance and constitutive defenses (density of resin canals and sclereids) were estimated according to Wright (1976):

$$[4] \quad h^2_i = 2\sigma^2_f / (\sigma^2_f + \sigma^2_e)$$

$$[5] \quad h^2_f = \sigma^2_f / (\sigma^2_f + \sigma^2_e/nb)$$

where σ^2_f is family variance, σ^2_e is the residual variance, n is the number of trees per family and block (n = 1) and b is the number of blocks (b = 30 for weevil resistance and b = 5 for constitutive defenses).

Pearson correlations were used to evaluate family relationships between weevil damage (percent of trees with leader kill) and constitutive resistance of Sitka spruce (density of resin canals and sclereid cells). These analyses were conducted on controlled-cross progeny means (N = 20).

Results

Weevil resistance

Following weevil release in the F1 progeny trial, overall mean attack rate increased from 0.5% (±0.1) (cumulative attack from 2004 to 2007) to 18.7% (±2.1) in 2008. After that, overall weevil attack rate decreased to 12.8% (±1.5) in 2009.

Weevil attack level varied at the full-sib family level (controlled-cross progeny). Weevil attack ranged from 0 to 52% among R × R crosses, from 16 to 83% among R × S

crosses and from 52 to 96% among S × S crosses (Table 1). The ten most weevil resistant crosses were R × R crosses and the ten least resistant crosses were either R × S or S × S crosses (Table 1).

The statistical analysis indicated significant differences in weevil attack among resistance cross level, parent provenances and controlled-cross families (Table 2). Specifically, considering the entire study period, there were significantly fewer leader kills among the R × R progeny, relative to the R × S or the S × S progeny (Figure 2). We also observed significantly fewer leader kills among the R × S progeny than in the S × S progeny (Figure 2). No significant differences in total height among resistance levels were observed in 2008, one year after weevil release ($F_{2,3031} = 0.21$, $P>0.05$), indicating that tree growth did not appear to be a factor for weevil preference.

Considering the provenances of parent trees, H × H, BQ × BQ and H × BQ (progeny obtained by crossing R × R parents) showed a percent of leader kill lower than the BQ × QCI crosses (progenie from R × S parents) and the QCI × QCI (progeny from S × S parents) (Figure 2). No significant differences were observed for the percent of leader kill between the provenance of resistant crosses (H × H, BQ × BQ and H × BQ parents) (Figure 2). Among the R × R progeny the controlled-crosses developed using a Haney-898 parent are of great interest due to their history of high weevil-resistance (Ying 1991; King and Alfaro 2009). Interestingly, 7 out of the 10 most resistant F1 crosses had the genotype 898 among its parents, either male or female (Table 1).

The estimated heritability on a family mean basis was high for weevil damage (h^2_f = 0.9), suggesting that important genetic gain in weevil resistance is possible through family selection. Individual heritability was also high ($h^2_i = 0.5$).

Constitutive defenses

283 Length and diameter of the terminal leaders used for histological analysis were not
284 significantly different among resistance levels ($F_{2,76}$ = 0.80, P>0.05 and $F_{2,76}$ = 2.29, P>0.05,
285 respectively).

286 Results from the mixed model indicated significant differences between resistance
287 levels for inner, outer and total resin canal density measured either by the number of resin
288 canals per square millimeter of bark or by the percentage of bark area occupied by resin canals
289 (Table 3). Specifically, the R × R F1 progeny had significantly greater density of inner, outer
290 and total resin canals than F1 progeny of the other two resistance levels (R × S and S × S)
291 (Table 4).

292 Results from ANOVA also indicated significant differences between parent
293 provenances for inner, outer and total resin canal density (Table 3). Especially, controlled-
294 crosses with both parents from Haney provenance showed significantly greater density of resin
295 canals (Table 4). Significant differences among controlled-cross progenies were observed for
296 outer and total resin canal density measured by the percentage of bark area occupied by resin
297 canals (Table 3). Significant differences among resistance levels and controlled-cross
298 progenies were also observed for sclereid cell density (Table 3). Specifically, the R × R
299 progeny had significantly greater density of sclereids (0.26 ± 0.03 sclereids mm^{-2}) than did
300 progeny from the other two resistance levels (0.14 ± 0.04 and 0.12 ± 0.05 sclereids mm^{-2} for R
301 × S and S × S progenies, respectively). However, no significant differences among parent
302 provenances were observed for sclereid cell density (Table 3). The estimated heritability on a
303 family mean basis was high (h^2_f = 0.8) for density of resin canals and for sclereids. Individual
304 heritability was moderate (h^2_i = 0.2) for both traits.

305 We found a positive and significant correlation at the family level between density of
306 outer and density of inner resin canal (Pearson r = 0.78, P < 0.001 for the density measured by
307 the number of resin canals per square millimeter of bark, and Pearson r = 0.62, P = 0.003 for

the density measured by the percentage of bark area occupied by resin canals). We also found a significant and positive family correlation between density of total resin canals and density of sclereid cells (Pearson r = 0.57, $P = 0.009$, N = 20 controlled-cross progenies).

The level of weevil damage was reduced in genotypes with high density of resin canals or sclereid cells. Significant and negative correlations were detected at the family (controlled-cross) level between the percentages of weevil attack and the average density of (i) inner resin canals (Pearson r = -0.64, $P = 0.004$), (ii) outer resin canals (Pearson r = -0.66, $P = 0.001$), (iii) total resin canals (Pearson r = -0.61, $P = 0.005$) and (iv) sclereid cell density (Pearson r = -0.48, $P = 0.032$) expressed by the number of each of them per square millimeter of bark.

Discussion

Weevil resistance

This study showed important genetic variation for white pine weevil resistance among Sitka spruce families. The weevil showed strong preference for some Sitka spruce families – particularly those from the QCI source. Our results showed that progeny from the R × R controlled-crosses had significantly higher weevil resistance compared to crosses with two susceptible parents (S × S), whereas the R × S progeny tended to have intermediate levels of weevil resistance, as would be expected under an additive genetic model. These results confirmed previous observations by Kiss and Yanchuk (1991), King et al. (1997) and Alfaro et al. (2004) for other spruce species.

Our results from the F1's confirm the strong provenance-based resistance that has long been observed (Alfaro and Ying 1990; King and Alfaro 2009; King et al. 2011). It has been postulated that extreme selection pressure in high weevil hazard zones increased the proportion of resistant trees in these areas, but it has been noted that resistance is not widely distributed throughout these high weevil hazard zones (King et al. 2004; King and Alfaro 2009). The high

susceptibility of the QCI provenance is probably due to the fact that *P. strobi* does not occur on the Queen Charlotte Islands and therefore natural selection for weevil resistance has not taken place within this provenance.

We also observed differences in weevil resistance among and within the resistant controlled-cross families, within parent provenances. For example, the high resistance of progenies from parents of the Haney genotype number 898 (Table 1) demonstrated the heritability of weevil resistance, since this parent was already known to be highly resistant to weevil from previous studies (King and Alfaro 2009). Individual and family heritability was high (0.5 and 0.9, respectively) suggesting that important gains can be expected through family selection. These heritabilities are generally higher than previous reports for spruce resistance to white pine weevil or other insects. Individual heritability for interior spruce resistance to *P. strobi* was found to be around 0.4 (King et al. 1997). Zas et al. (2005) observed that individual heritability for *Pinus pinaster* Ait. resistance to the weevil *Hylobius abietis* L. was around 0.2. Thus, our results confirmed that it is possible to develop a screening program to breed for Sitka spruce for resistance to the white pine weevil using individual putatively resistant parents to create highly resistant pedigreed progenies.

Constitutive defenses

Our results showed that controlled-crosses from R × R parents had greater density of resin canals and sclereid cells than controlled-crosses from R × S and S × S parents. Moreover, we found a negative relationship between the level of weevil attack by cross and constitutive defenses (density of resin canals and sclereid cells); that is, controlled-cross families which sustained fewer attacks had greater density of these constitutive defenses. These results support previous findings in a F1 progeny trial of interior spruce studied by Alfaro et al. (2004) who

found that weevil resistance was strongly correlated to constitutive defenses, and highlights the relevance of these traits for weevil resistance within the spruce genus.

We also observed large differences among parent provenances for the density of inner, outer and total resin canals. Controlled-crosses with both parents from Haney provenance (H × H) showed the greatest density of resin canals. Interestingly, the R × R controlled-crosses of Sitka spruce with both parents from Haney provenance showed density of total resin canals nearly 50% greater than R × R controlled-crosses with both parents from Big Qualicum resistant provenance, although they showed similar levels of weevil resistance. These results are in agreement with previous findings by Tomlin and Borden (1994) who found that half-sib families from the Big Qualicum provenance showed high resistance to white pine weevil, but relatively low density of outer resin canals. The high resistance of our controlled-crosses from Big Qualicum, which have lower density of constitutive resin canals and sclereid cells, indicates that these genotypes may rely more on other types of resistance mechanisms to defend against weevil attack, such as the content of phenolic or specific terpenoid compounds in the bark (Franceschi et al. 2000) or the induced production of traumatic resin canals (Alfaro 1995). Tomlin et al. (1997) and Robert et al. (2010) reported that resistant trees varied in levels of terpenoid compounds. Further studies of these additional resistance mechanisms are necessary to confirm our hypothesis.

In summary, results of this study indicate that screening of F1 Sitka spruce progeny for white pine weevil resistance can be used for recurrent selection of parents within existing seed orchards of the Sitka spruce tree improvement program, or to produce progeny for direct utilization in reforestation. Genetic resistance should be considered as an important strategy for an Integrated Pest Management System against the white pine weevil to help bring back Sitka spruce to the managed forests of western North America.

Acknowledgements

Authors thank Yvonne Sui, Jessie Simpson, Danielle Francis, Jodi Krakowski and Tony Ibaraki for help with field procedures and plant tissue collections. We also thank Terry Holmes for assistance with the histological analysis and Paul Boudewyn for help with statistical analysis. Comments and suggestions by Dr. Luis Sampedro and Dr. Rafael Zas helped to improve the manuscript. This work was completed during a research stay by the first author at the Pacific Forestry Centre (Victoria, British Columbia, Canada).

References

Alfaro, R.I. 1989 Stem defects in Sitka spruce induced by Sitka spruce weevil, *Pissodes strobi* (Peck). In *Proceedings of a Meeting of the IUFRO Working Group on Insects Affecting Reforestation: Biology and Damage held under the Auspices of the XVIII International Congress of Entomology*. Alfaro, R.I. and Glover, S.G. (eds). Vancouver, British Columbia, Canada, pp. 167–176.

Alfaro, R.I. 1995 An induced defense reaction in white spruce to attack by the white pine weevil *Pissodes strobi. Can. J. For. Res.* 25, 1725-1730.

Alfaro, R.I., Borden, J.H., Fraser, R.G. and Yanchuk, A. 1995 The white pine weevil in British Columbia: Basis for an integrated pest management system. *For. Chron.* 71, 66-73.

Alfaro, R.I., Borden, J.H., King, J.N., Tomlin, E.S., McIntosh, R.L. and Bohlmann J. 2002 Mechanisms of resistance in conifers against shoot infesting insects. In *Mechanisms and deployment of resistance in trees to insects*. M.R.Wagner, K.M. Clancy, F. Lieutier and T.D. Paine (eds). Kluwer Academic Publisher, Dordnecht, pp. 101-126.

Alfaro, R.I., He, F., Tomlin, E.T. and Kiss, G. 1997 White spruce resistance to white pine weevil related to bark resin canal density. *Can. J. Bot.* 75, 568-573.

Alfaro, R.I., He, F., Kiss, G., King, J.N. and Yanchuk, A. 1996 Resistance of white spruce to white pine weevil: development of a resistance index. *For. Ecol. Manage.* 81, 51-62.

408 Alfaro, R.I., King, J.N., Brown, R.G. and Buddingh, S.M. 2008 Screening of Sitka spruce
409 genotypes for resistance to the white pine weevil using artificial infestations. *For. Ecol.*
410 *Manage.* 255, 1748-1758.
411 Alfaro, R.I., Lewis, K.G., King, J.N., El Kassaby, Y.A., Brown, G. and Smith, L.D. 2000
412 Budburst phenology of Sitka spruce and its relationship to white pine weevil attack.
413 *For. Ecol. Manage.* 127, 19-29.
414 Alfaro, R.I., vanAkker, L., Jaquish, B. and King, J.N. 2004 Weevil resistance of progeny
415 derived from putatively resistant and susceptible interior spruce parents. *For. Ecol.*
416 *Manage.* 202, 369–377.
417 Alfaro, R.I. and Ying, C. 1990 Levels of Sitka spruce weevil, *Pissodes strobi* (Peck), damage
418 among Sitka spruce provenances and families near sayward, British Columbia. *Can.*
419 *Entomol.* 122, 607-615.
420 Cassab, G.I. 1998 Plant cell wall proteins. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 281-
421 309.
422 Franceschi, V., Krokene, P. and Krekling, T. 2005 Anatomical and chemical defenses of
423 conifer bark against bark beetles and other pests. *New Phytol.* 167, 353–376.
424 Franceschi, V., Krokene, P. and Krekling, T. and Christiansen, E. 2000 Phloem parenchyma
425 cells are involved in local and distant defense responses to fungal inoculation or bark-
426 beetle attack in Norway spruce (*Pinaceae*). *Am. J. Bot.* 37, 314–326.
427 Gerson, E.A., Kelsey, R.G. and St Clair, J.B. 2009 Genetic variation of piperidine alkaloids in
428 *Pinus ponderosa*: a common garden study. *Ann. Bot.* 103, 447-457.
429 He, F. and Alfaro, R.I. 2000 White pine weevil attack on white spruce: a survival time
430 analysis. *Ecol. Appl.* 10, 225-232.
431 King, J.N. and Alfaro, R.I. 2001 Screening of Sitka spruce genotypes for resistance to the
432 white pine weevil in British Columbia. In *Genetic response of forest systems to*

433 *changing environmental conditions*. G. Müller-Stark and R. Schubert (eds). Kluwer
434 Academic Publisher, Dordrecht, Netherlands, pp. 11–20.
435 King, J.N. and Alfaro, R.I. 2009 Developing Sitka spruce populations for resistance to the
436 white pine weevil: Summary of research and breeding program. *B.C. Ministry of*
437 *Forests and Range, Research Branch.* 050. available at: http://
438 www.for.gov.bc.ca/hfd/pubs/docs/Tr/Tr050.pdf
439 King, J.N., Yanchuk, A., Kiss, G. and Alfaro, R.I. 1997 Genetic and phenotypic relationships
440 between weevil (*Pissodes strobi*) resistance and height growth in spruce populations of
441 British Columbia. *Can. J. For. Res.* 27, 732-739.
442 King, J.N. and Alfaro, R.I. and Cartwright, C. 2004 Genetic resistance of Sitka spruce (*Picea*
443 *sitchensis*) populations to the white pine weevil (*Pissodes strobi*): distribution of
444 resistance. *Forestry.* 77, 269-278.
445 King, J.N., Alfaro, R.I., Grau Lopez, M. and VanAkker, L. 2011 Resistance of Sitka spruce
446 (*Picea sitchensis*) to white pine weevil (*Pissodes strobi*): characterizing the bark
447 defence mechanisms of resistant populations. *Forestry.* 84, 83-91.
448 Kiss, G. and Yanchuk, A. 1991 Preliminary evaluation of genetic variation of weevil resistance
449 in interior spruce in British Columbia. *Can. J. For. Res.* 21, 230-234.
450 Klinka, K., Pojar, J. and Meidinger, D.V. 1991 Revision of biogeoclimatic units of coastal
451 British Columbia. *Northwest. Sci.* 65, 32-47.
452 Littell, R.C., Milliken, G.A., Stroup, W.W., Wolfinger, R. and Schabenberger, O. 2006 *SAS*®
453 *System for Mixed Models*. SAS Institute, Cary, NC.
454 Mitchell, R.G., Wright, K.H. and Johnson, N.E. 1990 Damage by the Sitka spruce weevil
455 (*Pissodes strobi*) and growth patterns for 10 spruce species and hybrids over 26 years in
456 the Pacific Northwest. *US Dep. Agric. For.Serv. Res. Paper* PNW-RP-434.

457 Moreira, X., Sampedro, L., Zas, R. and Solla, A. 2008 Alterations of the resin canal system of
458 *Pinus pinaster* seedlings after fertilization of a healthy and of a *Hylobius abietis*
459 attacked stand. *Trees*. 22, 771-777.
460 Núñez-Farfán, J., Fornoni, J. and Valverde, P.L. 2007 The evolution of resistance and tolerance
461 to herbivores. *Ann. Rev. Ecol. Evol. Syst.* 38, 541-566.
462 Phillips, M.A. and Croteau, R.B. 1999 Resin-based defenses in conifers. *Trends Plant Sci.* 4,
463 184-190.
464 Roberds, J.H., Strom, B.L., Hain, F.P., Gwaze, D.P., McKeand, S.E. and Lott, L.H. 2003
465 Estimates of genetic parameters for oleoresin and growth traits in juvenile loblolly pine.
466 *Can. J. For. Res.* 33, 2469–2476.
467 Robert, J.A., Madilao, L.L., White, J., Yanchuk, A., King, J.N., Bohlmann, J. 2010 Terpenoid
468 metabolite profiling in Sitka spruce identifies association of dehydroabietic acid, (+)-3-
469 carene and terpinolene with resistance against the white pine weevil. *Can. J. Bot.* 88,
470 810-820.
471 Rosner, S. and Hannrup, B. 2004 Resin canal traits relevant for constitutive resistance of
472 Norway spruce against bark beetles: environmental and genetic variability. *For. Ecol.*
473 *Manage.* 200, 77-87.
474 Ruzin, S.E. 1999 *Plant microtechnique and microscopy*. Oxford University Press, New York.
475 Silver, G.T. 1968 Studies on the Sitka spruce weevil, *Pissodes sitchensis*, in British Columbia.
476 *Can. Entomol.* 100, 93-110.
477 Soil Classification Working Group 1998 *The Canadian System of Soil Classification*. Agric.
478 and Agri-Food Can. Publ. 1646, NRC Research Press, Ottawa.
479 Taylor, S., Alfaro, R.I., Delong, C. and Rankin, L. 1996 The effects of overstory shading on
480 white pine weevil damage to interior white spruce and its effects on growth rates. *Can.*
481 *J. For. Res.* 26, 306-312.

Tomlin, E.S. and Borden, J.H. 1994 Relationship between leader morphology and resistance or susceptibility of Sitka spruce to the white pine weevil. *Can. J. For. Res.* 24, 810-816.

Tomlin, E.S., Borden, J.H. and Pierce, H.D. 1997 Relationship between volatile foliar terpenes and resistance of Sitka spruce to the white pine weevil. *For. Sci.* 43, 501-508.

Trapp, S. and Croteau, R. 2001 Defensive resin biosynthesis in conifers. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 52, 689-724.

vanAkker, L., Alfaro, R.I. and Brockley, R. 2004 Effects of fertilization on resin canal defences and incidence of *Pissodes strobi* attack in interior spruce. *Can. J. For. Res.* 34, 855-862.

Wainhouse, D. and Ashburner, R. 1996 The influence of genetic and environmental factors on a quantitative defensive trait in spruce. *Funct. Ecol.* 10, 137-143.

Wright, J.W. 1976 *Introduction to forest genetics*. Academic Press, NewYork.

Ying, C.C. 1991 *Genetic resistance to the white pine weevil in Sitka Spruce. B.C. Ministry of Forests Res. Note No.* 106. Victoria, BC.

Zas, R., Sampedro, L., Prada, E., Fernández-López, J. 2005 Genetic variation of *Pinus pinaster* Ait. seedlings in susceptibility to the pine weevil *Hylobius abietis* L. *Ann. For. Sci.* 62, 681-688.

505 **Table 1.** Resistance ranking of white pine weevil for progeny obtained by controlled crosses of
506 putatively resistant and susceptible Sitka spruce. Crosses developed using parent 898, a well-
507 known resistant parent, are presented in bold.
508

Resistance ranking	Cross	Male parent	Female parent	Provenance (male x female) [1]	Resistance (male x female) [2]	Cumulative attack of F1 progeny (%)
1	31	1179	**898**	H x H	R x R	0.0
2	56	889	1172	H x H	R x R	0.0
3	57	1172	1172	H x H	R x R	0.0
4	61	1253	**898**	H x H	R x R	3.3
5	72	**898**	1020	H x BQ	R x R	3.3
6	30	1152	**898**	H x H	R x R	3.5
7	38	**898**	1179	H x H	R x R	3.6
8	67	855	868	BQ x BQ	R x R	3.6
9	29	1139	**898**	H x H	R x R	6.7
10	41	**898**	1209	H x H	R x R	6.7
11	44	1210	1209	H x H	R x R	6.7
12	58	**898**	848	H x BQ	R x R	6.7
13	63	889	1018	H x BQ	R x R	6.7
14	51	1172	1156	H x H	R x R	6.9
15	71	1152	889	H x H	R x R	6.9
16	81	1253	1114	H x BQ	R x R	6.9
17	2	855	848	BQ x BQ	R x R	7.1
18	75	868	1020	BQ x BQ	R x R	7.1
19	16	868	860	BQ x BQ	R x R	7.4
20	40	1210	1179	H x H	R x R	10.0
21	52	1253	1156	H x H	R x R	10.0
22	60	889	**898**	H x H	R x R	10.0
23	62	**898**	866	H x BQ	R x R	10.0
24	77	868	1187	H x BQ	R x R	10.0
25	45	1156	889	H x H	R x R	10.3
26	46	889	1075	H x H	R x R	10.3
27	73	868	892	H x BQ	R x R	10.3
28	37	1210	1152	H x H	R x R	10.7
29	22	862	868	BQ x BQ	R x R	13.3
30	32	**898**	1139	H x H	R x R	13.3
31	49	1253	1075	H x H	R x R	13.3
32	83	1156	1178	H x H	R x R	13.3
33	85	1139	1158	H x H	R x R	13.3
34	48	1159	1075	H x H	R x R	13.8
35	68	849	1234	BQ x BQ	R x R	13.8

36	70	866	1210	H x BQ	R x R	13.8
37	53	889	1159	H x H	R x R	14.3
38	50	1159	1156	H x H	R x R	16.0
39	93	1139	209	H x QCI	R x S	16.1
40	19	1226	862	BQ x BQ	R x R	16.7
41	59	1253	848	H x BQ	R x R	16.7
42	64	1253	866	H x BQ	R x R	16.7
43	21	860	868	BQ x BQ	R x R	17.2
44	43	1152	1209	H x H	R x R	17.2
45	54	1172	1159	H x H	R x R	17.2
46	82	868	1178	H x BQ	R x R	17.2
47	24	860	872	BQ x BQ	R x R	17.9
48	6	849	855	BQ x BQ	R x R	18.5
49	8	866	855	BQ x BQ	R x R	20.0
50	11	866	856	BQ x BQ	R x R	20.0
51	35	1210	1139	H x H	R x R	20.0
52	10	855	856	BQ x BQ	R x R	20.7
53	47	1156	1075	H x H	R x R	20.7
54	84	1253	1178	H x H	R x R	20.7
55	65	1253	849	H x BQ	R x R	21.4
56	99	**898**	259	H x QCI	R x S	21.4
57	4	856	849	BQ x BQ	R x R	23.3
58	66	889	1139	H x H	R x R	23.3
59	7	856	855	BQ x BQ	R x R	24.1
60	20	1234	862	BQ x BQ	R x R	24.1
61	78	868	1241	BQ x BQ	R x R	24.1
62	79	866	1167	H x BQ	R x R	24.1
63	87	866	1020	BQ x BQ	R x R	24.1
64	97	1210	253	H x QCI	R x S	25.9
65	3	866	848	BQ x BQ	R x R	26.7
66	14	866	1018	BQ x BQ	R x R	26.7
67	23	1234	868	BQ x BQ	R x R	26.7
68	26	868	872	BQ x BQ	R x R	26.7
69	80	866	1153	H x BQ	R x R	26.7
70	12	848	1018	BQ x BQ	R x R	27.6
71	55	1253	1159	H x H	R x R	27.6
72	15	862	860	BQ x BQ	R x R	28.6
73	17	860	862	BQ x BQ	R x R	29.6
74	86	849	1007	BQ x BQ	R x R	29.6
75	9	848	856	BQ x BQ	R x R	30.0
76	27	1234	872	BQ x BQ	R x R	30.0
77	28	860	1234	BQ x BQ	R x R	30.0
78	18	868	862	BQ x BQ	R x R	31.0
79	25	862	872	BQ x BQ	R x R	31.0
80	13	849	1018	BQ x BQ	R x R	32.1

81	36	1139	1152	H x H	R x R	33.3
82	95	**898**	249	H x QCI	R x S	33.3
83	42	1139	1209	H x H	R x R	34.5
84	69	848	1139	H x BQ	R x R	34.5
85	34	1179	1139	H x H	R x R	37.9
86	96	**898**	256	H x QCI	R x S	37.9
87	1	849	848	BQ x BQ	R x R	41.4
88	39	1139	1179	H x H	R x R	43.3
89	88	866	1151	H x BQ	R x R	43.3
90	98	1253	253	H x QCI	R x S	46.7
91	74	862	1007	BQ x BQ	R x R	48.2
92	5	866	849	BQ x BQ	R x R	48.3
93	33	1152	1139	H x H	R x R	50.0
94	91	1018	259	BQ x QCI	R x S	50.0
95	76	1152	915	H x H	R x R	51.9
96	106	259	259	QCI x QCI	S x S	51.9
97	101	1253	259	H x QCI	R x S	53.6
98	94	848	209	BQ x QCI	R x S	55.2
99	100	1210	259	H x QCI	R x S	70.0
100	90	866	259	BQ x QCI	R x S	71.4
101	89	866	253	BQ x QCI	R x S	72.4
102	107	259	259	QCI x QCI	S x S	79.3
103	108	259	316	QCI x QCI	S x S	79.3
104	102	253	249	QCI x QCI	S x S	80.0
105	105	249	253	QCI x QCI	S x S	82.1
106	103	259	249	QCI x QCI	S x S	82.8
107	92	849	237	BQ x QCI	R x S	83.3
108	109	259	316	QCI x QCI	S x S	85.2
109	104	249	253	QCI x QCI	S x S	90.0
110	110	316	316	QCI x QCI	S x S	96.2

[(1)] H = Haney provenance parents (resistant), BQ = Big Qualicum River provenance parents (resistant) and QCI = Queen Charlotte Islands provenance parents (susceptible).

[(2)] R = resistant parent, S = susceptible parent.

Table 2. Summary of the mixed model used to evaluate white pine weevil resistance (noted as the presence or absence of attack from 2004 to 2009) of the progeny obtained by controlled crosses of putatively resistant and susceptible Sitka spruce. Significance (P<0.05) P values are typed in bold.

	DF[1]	F	*P*
Block	29, 3051	2.06	**0.001**
Resistance	2, 3051	25.93	**<0.001**
Provenance[2]	4, 3051	11.62	**<0.001**
Cross[3]	103, 3051	2.14	**<0.001**

[1]DF = degrees of freedom (numerator, denominator)

[2]Provenance is nested within resistance

[3]Controlled-cross is nested within provenance and resistance

Table 3. Significance tests on resin canal and sclereid cell density by resistance class, provenance and control-pollinated families. Significant (P<0.05) P values are in bold.

		Inner resin canal				Outer resin canals				Total resin canals				Sclereids	
		Number		Percentage		Number		Percentage		Number		Percentage		Number	
Effect	DF[1]	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Resistance	2, 76	4.18	**0.010**	2.96	**0.046**	3.80	**0.027**	2.16	0.123	3.92	**0.024**	3.02	**0.047**	3.68	**0.029**
Provenance[2]	2, 76	5.55	**0.006**	3.34	**0.042**	5.92	**0.004**	3.17	**0.044**	5.97	**0.004**	3.91	**0.025**	2.29	0.108
Cross[3]	15, 76	1.11	0.359	2.60	**0.004**	1.37	0.183	0.87	0.597	1.15	0.326	2.62	**0.004**	1.70	**0.044**
Leader diameter[4]	1, 76	15.02	**<0.001**	0.01	0.942	8.02	**0.006**	2.61	0.111	15.82	**<0.001**	0.21	0.645	1.49	0.225

[1]DF = degrees of freedom (numerator, denominator)

[2]Provenance is nested within resistance

[3]Controlled-cross is nested within provenance and resistance

[4]Diameter of the terminal leaders was used as covariate

553 Table 4. Least-square means of resin canal density by resistance classes and provenances.
554 Different letters indicate significant differences at $P<0.05$.
555

	Inner resin canals		Outer resin canals		Total resin canals	
Resistance level[1]	Number	Percentage	Number	Percentage	Number	Percentage
R × R	2.10 a	2.98 a	1.60 a	0.82 a	3.73 a	3.81 a
R × S	1.59 b	2.72 ab	1.19 b	0.68 a	2.80 b	3.46 b
S × S	1.51 b	2.36 b	1.10 b	0.61 a	2.60 b	3.14 b

	Inner resin canals		Outer resin canals		Total resin canals	
Parent provenance[2]	Number	Percentage	Number	Percentage	Number	Percentage
H × H	2.51 a	3.06 a	1.99 a	0.95 a	4.51 a	4.01 a
BQ × BQ	1.68 b	2.91 ab	1.22 b	0.69 b	2.94 b	3.61 ab
H × QCI	1.71 b	3.04 a	1.36 b	0.77 ab	3.07 b	3.91 a
BQ × QCI	1.47 b	2.41 bc	1.03 b	0.60 b	2.52 b	3.01 c
QCI × QCI	1.51b	2.36 c	1.10 b	0.61 b	3.00 b	3.14 bc

556

557 [1]R × R= resistant parent crossed with resistant parent, R × S = resistant parent crossed with
558 susceptible parent, S × S = susceptible parent crossed with susceptible parent.
559 [2]Haney (H) and Big Qualicum (BQ) provenances are considered as weevil resistant while
560 Queen Charlotte Island (QCI) provenance is considered as weevil susceptible.
561
562
563
564
565
566

FIGURE LEGENDS

Figure 1. Cross-section of a Sitka spruce terminal leader, indicating inner and outer resin canals and showing a group of sclereid cells adjacent to a vascular bundle.

Figure 2. Percent of trees with top-kill resulting from attack by *Pissodes strobi,* in Sitka spruce progeny from controlled crosses of putative weevil resistant and susceptible parents (cumulative attack from 2004 to 2009). Codes for the parent resistant level are: R = resistant parent, S = susceptible parent. Codes for the parent provenance are: H = Haney provenance parents (resistant), BQ = Big Qualicum River provenance parents (resistant) and QCI = Queen Charlotte Islands provenance parents (susceptible).

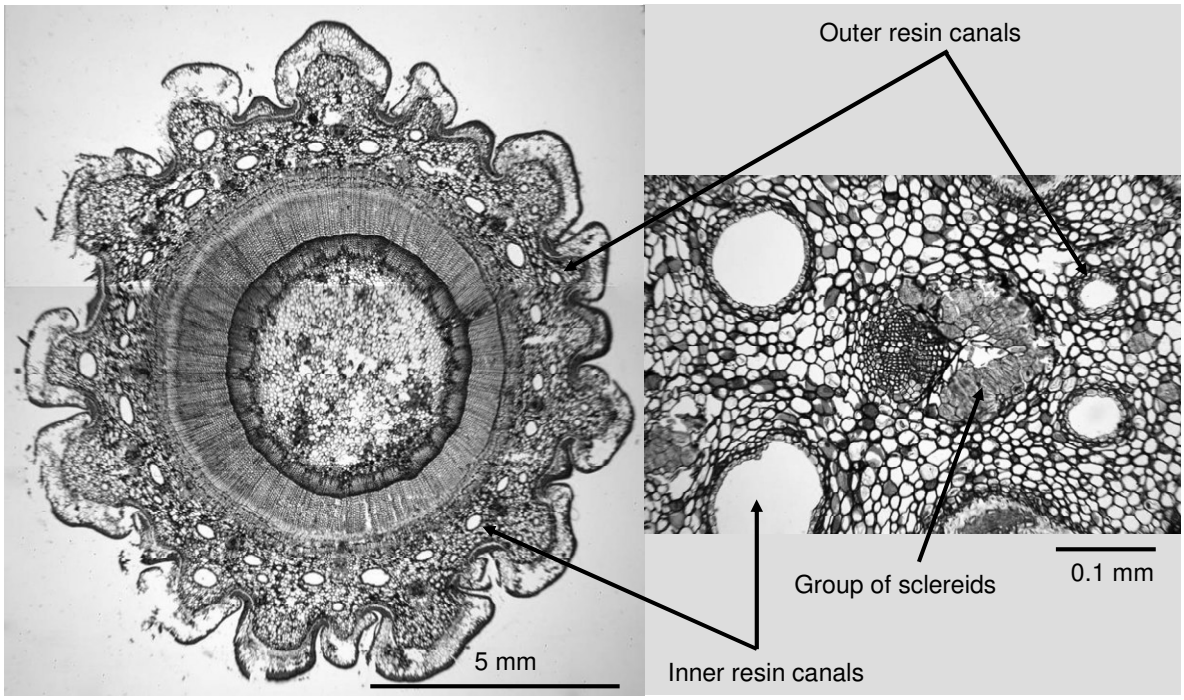

Figure 1. Moreira et al.

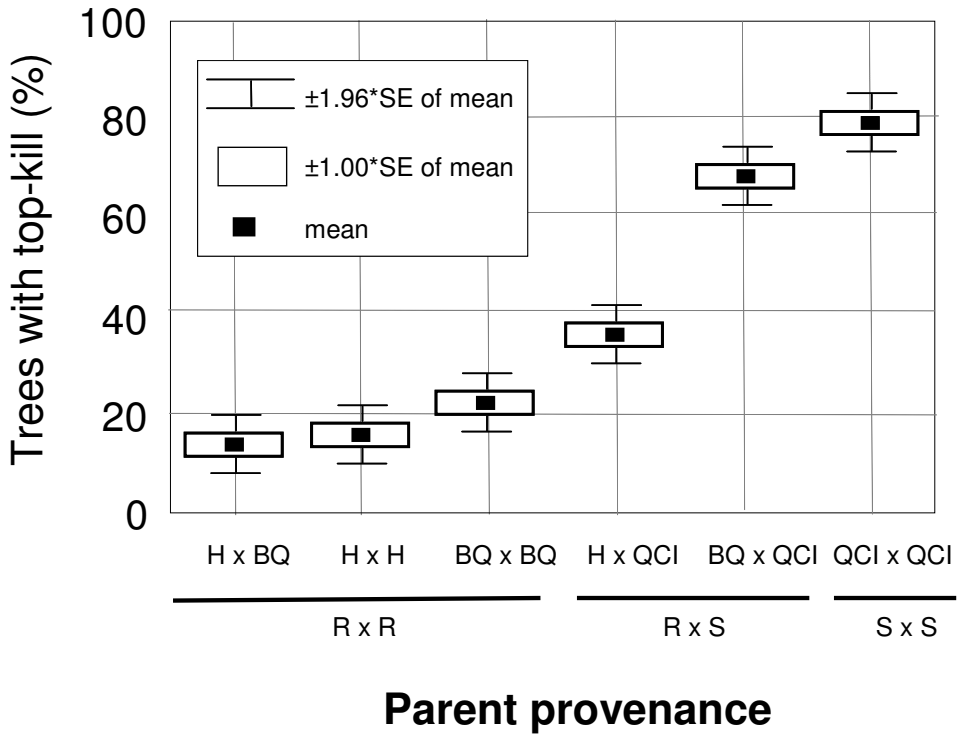

Figure 2. Moreira et al.